Communications to the Editor

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THE β-GLUCOSIDATION AND β-GLUCURONIDATION OF PANTOTHENIC ACID COMPARED WITH P-NITROPHENOL IN DOG LIVER MICROSOME

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The β-glucosidation and β-glucuronidation of pantothenic acid (PaA) in dog liver microsome were compared with those of p-nitrophenol. PaA underwent β-glucosidation in the presence of uridine diphosphate glucose (UDPG). However, p-nitrophenol did not conjugate β-glucose but underwent β-glucuronidation in the presence of uridine diphosphate glucuronic acid (UDPGA). The UDP-glucuronyltransferase effect on p-nitrophenol was 1000 times larger than the UDP-glucosyltransferase effect on PaA. But no glucuronidation of PaA occurred. The optimum pH for the glucosidation of PaA was 8.5. These results indicate that PaA has a high specificity for β-glucosidation, and that β-glucosidation is an essential pathway in the metabolism of PaA in dogs.

KEYWORDS——pantothenic acid; p-nitrophenol; β-glucosidation; β-glucuronidation; dog liver microsome

We have reported that dogs excrete PaA β-glucoside as the only metabolite of PaA, and that in dog microsomal preparations glucose was transferred from UDPG to PaA to form PaA β-glucoside.[1)] To our knowledge, this is the first demonstration of the occurrence of PaA glucoside in mammals. Although glucoside formation in mammals has been demonstrated for estradiols,[2)] bilirubin,[3)] riboflavine,[4)] and some xenobiotics,[5)] these compounds are preferentially glucuronidated. It has been suggested that the glucoside formation is an alternative pathway operative only in the absence of an adequate amount of UDPGA.[5b)] In contrast, PaA β-glucoside in the dogs was the only urinary metabolite of PaA, accounting for up to 40% of the radioactivity excreted in 24 h.[1)] Such an exclusive formation of β-glucoside is peculiar to mammals. However, the possibility remained that PaA glucuronide was formed but could not be detected in urine because of hydrolysis. Therefore, in the present investigation we compared β-glucosidation and β-glucuronidation of PaA with those of p-nitrophenol using dog liver microsomes to determine whether or not PaA undergoes β-glucuronidation.

Four beagle dogs were used. Liver microsomes were prepared as described in the previous paper.[1)] In order to determine the UDP-glucosyltransferase activity, 1 mM ^{14}C-PaA (1 mCi/mmole, New England Nuclear) was incubated with 10 mM UDPG in the medium containing 1-2 mg of dog liver microsomal protein, 5 mM $MgCl_2$, 100 mM Tris-HCl (pH 7.4) in a total volume of 0.5 ml at 37°C for 60 min. The UDP-glucosyl-

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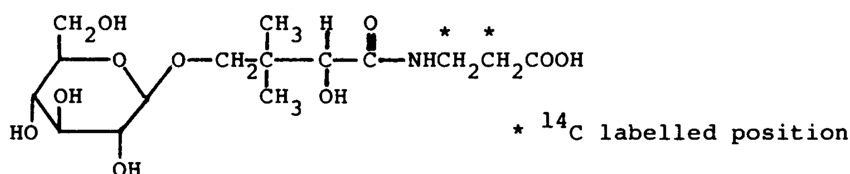

Fig. 1. 4'-O-(β-D-Glucosyl)-D-pantothenic Acid (PaA β-Glucoside)

transferase effect on PaA was determined from the production rate of PaA β-glucoside. This was monitored by HPLC using a Radial Pak C_{18} column with the solvent of formic acid/methanol (95:5, v/v) adjusted to pH 2.9 with 1% triethylamine at a flow rate of 2 ml/min. The eluates from the column were collected into scintillation vials to measure radioactivity. Radioactive peaks appeared at 11 and 19 min and were identified by GC/MS analysis as PaA and PaA β-glucoside, respectively.[1)] The production of PaA β-glucoside increased linearly up to 90 min. Its rate was linearly dependent on the protein concentration of microsome (0.5 - 2 mg/ml). PaA β-glucoside was not formed in the absence of either microsomes or UDPG, and Mg^{++} was necessary to activate UDP-glucosyltransferase as it was for UDP-glucuronyltransferase.[6e)]

The rates of the conjugation reaction as a function of pH were measured in the presence of 0.1 M MES-NaOH (pH 5.8 - 7.0), 0.1 M Tris-HCl (pH 7.0 -8.2) or 0.1 M carbonate-bicarbonate (pH 8.2 -10.0). The rate of the glucoside formation as a function of pH is shown in Fig. 2. The optimum pH for the glucosyltransferase activity of rabbit liver is 7.0 for estradiol-17α and 8.0 for estradiol-17α 3-glucuronide.[2d)] The former is glucosidated at 3-phenolic OH, and the latter at 17-OH. The optimum pH for p-nitrophenol[5)] and bilirubin[3d)] is 7.0. In contrast, the optimum pH for PaA was 8.5, which was close to that for estradiol-17α 3-glucuronide described above. The inflection about pH 7.0 in Fig. 2 seems to correspond to the optimum pH for estradiol, p-nitrophenol and bilirubin.

Enzyme effects on PaA are shown in Table I. The inhibitory effects of bilirubin (0.05, 0.1, 0.2 mM) or p-nitrophenol (0.1, 0.5, 1 mM) on the formation of PaA β-glucoside were examined under the same incubation conditions as described above. These compounds are known to be glucosidated, but they did not affect the β-glucosidation of PaA (Table I).

It has been reported that UDP-glucuronyltransferase is activated by Triton X-100[6)] and UDP-N-acetylglucosamine.[7)] However, neither Triton X-100 (0.01%) nor UDP-N-acetylglucosamine (5 mM) activated the glucosidation of PaA in dog liver microsome.

Glucosidation of p-nitrophenol was examined by a colorimetric procedure[8)] under the same incubation conditions as above except that 1 mM p-nitrophenol was used instead of PaA. The optical density of the supernatant of the reaction mixture was read at 405 nm to measure the disappearance of p-nitrophenol upon glucosidation. No glucosidation proceeded with p-nitrophenol (Table I).

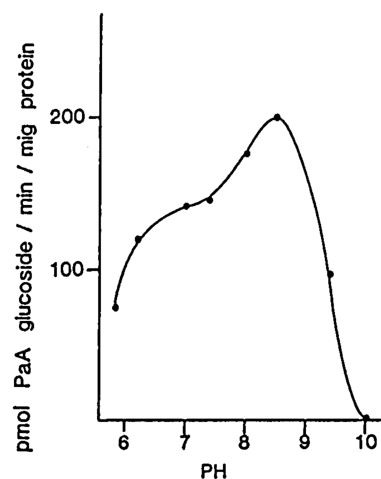

Fig. 2. Effect of pH on PaA β-Glucoside Formation

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P-Nitrophenol has been reported to be glucosidated by UDP-glucosyltransferase. However, the glucosidation was less active than glucuronidation in mouse liver microsomes,[5c)] and p-nitrophenol was a poorer substrate for glucosidation in rabbit liver than in mouse liver.[2d)] No p-nitrophenol β-glucoside formation in this preparation was probably a species-difference effect.

To examine the UDP-glucuronyltransferase effect on p-nitrophenol, 1 mM p-nitrophenol was incubated with 3 mM UDPGA, microsome (1 mg protein), 0.01% Triton X-100, 4 mM $MgCl_2$ in 100 mM Tris-HCl (pH 7.4) at 37°C for 15 min. The disappearance of p-nitrophenol upon glucuronidation was determined by the colorimetric method described above. p-Nitrophenol was well glucuronidated. The rate of β-glucuronidation of p-nitrophenol was about 1000 times higher than that of the β-glucosidation of PaA (Table I), suggesting the presence of large amounts of UDP-glucuronyltransferase in the preparation. Nevertheless, there was no β-glucuronidation of PaA. PaA (1 mM) had no effect on the glucuronidation of p-nitrophenol (1 mM).

Table I. UDP-glucosyltransferase and UDP-glucuronyltransferase effects on PaA and p-Nitrophenol in Liver Microsome of Beagle Dogs

Substrate	Enzyme activity	
	UDP-glucosyltransferase[a)]	UDP-glucuronyltransferase[a)]
PaA	0.0657 ± 0.0125	0
+ p-Nitrophenol (0.1 - 1 mM)	No effect	——
+ Bilirubin (0.1 - 0.2 mM)	No effect	——
p-Nitrophenol	0	76.5 ± 17.0
+ PaA (1 mM)	——	No effect

a) Results are expressed as mean ± S.D. (N = 4) of enzyme activities (nmol/min/mg protein) for 1 mM PaA or 1 mM p-nitrophenol.

Glucosidation usually occurs at acidic hydroxyl groups such as phenol or carboxylic acid.[5)] Estradiol has a phenol group at C-3 and a hydroxyl group at C-17. Collins et al. reported that glucose was conjugated to both groups of estradiol, but phenol-glucosidation was favored.[2b)] In contrast, glucosidation of PaA occurred exclusively at the 4'-hydroxyl group in spite of the presence of a carboxyl group (Fig. 1). P-Nitrophenol and bilirubin did not inhibit PaA glucosidation. This, together with the difference of the pH profile and the specificity for the non-acidic hydroxyl group, suggests that the UDP-glucosyltransferase effect on PaA is highly substrate specific, although further studies are needed to explore the mutiplicity of UDP-glucosyltransferase effects. The urinary metabolite of PaA in rat has been reported to be 4'-phosphopantothenic acid.[9)] In dogs, however, it was β-glucoside. It is of interest that the 4'-position, which is phosphorylated as a step to CoA synthesis, is also highly glucosidated. Although studies of species differences in PaA metabolism have not been reported, the glucosidation process found in dogs seems to have a special significance in the metabolism of PaA, considering the high amount and specificity of the formation of the metabolite.

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